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HEMATOLOGICAL STUDY OF *CHANNA MARULIUS* WITH REFERENCE TO TRYPANOSOMIASIS

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ABSTRACT: The present study deals with the description of fish infection of trypanosome species found infecting the fresh water fish *Channa marulius* from river Wainganga, Chandrapur District, Maharashtra, India, and evaluating the hematological parameters of the infected fish. Trypanosome is widespread parasite in freshwater fishes, causing disease trypanosomiasis. This study was implemented to verify the infection with *Trypanosoma sp.* report the cellular intervening through inflammatory response in blood smears of infected fishes. Present investigation deals with the hematological studies on *Channa marulius* an economically important freshwater fish, with reference to trypanosome infection ,75 % of *Channa marulius* population from Wainganga river system, showed trypanosome infection. This severe infection resulted decrease in total W.B.C count and total R.B.C. Significantly decreases Small lymphocytes count has shown due to infection. Also, there is co-relation between number of lymphocytes and various polymorphic stages of trypanosomes. Red dark circle, lesions on body, sluggishness in body movements are symptom of infection and swelling of body at pectoral fin region.

Key words: - Tryapanosome, Channa marulius, Hematology, Infection, Lymphocytes.

INTRODUCTION :

Fish is an excellent and one of the primary sources of protein for humans in many parts of the world. Fish is more affordable and accessible animal source food and it is estimated that around 60 % of people in many developing countries depend on fish, for over 30 % of their animal protein supplies (FAO 2005). Consumption will increase in future years as the demand will further be compounded by the growth in population and this additional supply will have to come from aquaculture (FAO 2012). Development of aquaculture during the last decade has resulted in much greater attention being paid to problems posed by parasites and their importance for fishery leading to constraints in the productivity of aquaculture (Kennedy 1994). Besides direct losses caused by mortality, parasites may have considerable impact on growth and behavior of fish, their resistance to other stress factors, susceptibility to predation, etc. and their presence may also reduce marketability of fish (Crowden and Boom 1980; Brassard et al. 1982; Lom and Dyková 1992; Williams and Jones 1994; Kumaraguru et al. 1995; Woo 1995).

Trypanosoma danilewskyi was first described from the blood of the common carp (*Cyprinus carpio*) in Europe (Laveran and Mesnil, 1904). The parasite has since been found in carp, goldfish (*Carassius auratus*), tench (*Tinca tinca*) and eel (*Anguilla* sp.) in Europe (Thompson, 1908; Pavlovskii, 1964; Lom, 1973) and in *Saccobranchus fossilis* in India (Qadri, 1962). Trypanosoma, a flagellate protozoan, is found in the blood of many vertebrates as parasite and causes a disease called trypanosomiasis in humans and livestock. Chronic infection with trypanosome dwelling extra-cellularly in blood and tissues of their hosts are observed in all vertebrate classes (Haag *et al.*, 1998). The genus Trypanosoma

(Trypanosomatidae: Kinetoplastida) comprises unicellular flagellate that are parasites of all vertebrate classes. The characteristic morphological forms of trypanosomes are tryptomastigotes observed in blood and tissues of vertebrate hosts and epimastigotes, which are found in invertebrate vector. The vectors can be hematophagous arthropods (insects or arachnids) for mammalian, avian, some of the amphibian and some of the reptilian trypanosomes while fish and certain other amphibian and reptilian trypanosomes are transmitted by leeches. Infective stages which develop from epimastigotes in vector are designated metacyclic forms they have tryptomastigote morphology (Haag *et al.,* 1998).

Channa marulius is native to South Asia. In South and Central India it is commonly found in reservoirs of eastern Vidarbha region. It is a faster growing fish than most of the other species of the genus. It is a carnivorous species. It is marketed live and fetches high prices in the market.

The fish *Channa marulius* is well known for its nutritional value. A well-known economic loss to the fish industry was the major outbreak of bacterial infection in major carps.The causative agents of the severe acute infectious abdominal dropsy outbreak in Indian major carps.*Cirrhinus mrigala* was reported by Shome et al (1996). However, the first observation on diseases in Indian major carps was found in descending order of susceptibility on *Catla catla*, *Cirrhinus mrigala* and *Labeo rohita* (Gopalakrishnan 1981). Other well recorded cases have been the severe epidemic due to the diseased condition of European carps (Snieszko 1954; Van Dujin 1956).

Trypanosoma probably is the best known extra erythrocytic protozoan in fish (Clauss et al. 2008) and undoubtedly represents one of the most important groups of pathogens which negatively affect the health state of cultured and feral fish. *Trypanosoma* are single celled flagellated protozoans that are generally found in the blood of vertebrates and can cause trypanosomiasis of fish, including fresh water fish (Smit et al. 2004; Davies et al. 2005; Padua et al. 2011).

The study of the physiological and hematological characteristics of cultured fish species is an important tool for the development of aquaculture system, particularly in regard to the use in detection of healthy fish from diseased or stressed animal (Rainza-paiva et al. 2000; O'Neal and Weirich 2001). Haematological studies are an important tool for diagnosis and prognosis of morbid conditions, including the deleterious effects of infection (Tavares-Dias and Moraes 2003). Biological monitoring techniques involving hematological variables have become attractive and useful for monitoring environmental quality, water pollution and the health condition of aquatic organisms (Kori-Siakpere and Ubogu 2008; Olufayo 2009; Qiang et al. 2013). The evaluation of haematological parameters could be useful for the diagnosis of fish pathologies and physiological status (Stoskopf 1993). Therefore, the variations of haematological parameters associated with various parasite infections could be established into a database, which could be used as biomarkers in disease diagnosis and in guiding the implementation of treatment or preventive measures. The studies related to haematological alterations in *Trypanosoma* infected fish are scanty (Joshi and Dabral 1981; Khan 1985; Islam and Woo 1991; Gupta and Gupta 2010) and further research is required involving diverse hosts, so that the haematological variations associated are elucidated and used as an efficient *Trypanosoma* infection indicator.

MATERIALS AND METHODS:

A total of 142 individuals of the freshwater fish *Channa marulius* were examined from the local fish markets. The sampled fish were measured

individually for standard length (L) and body weight (W). Fish blood samples were collected by decapitating of head of the fishes. Blood smears were fixed in absolute methanol for 5 minutes, were stained with Qualigen's Giemsa and were screened for the blood parasites with light microscopy at 1000x magnification. Parasites were measured using morphometry on random selection, according to terminology of morphometric values and standard commonly adapted by Hoare (1972). Morphometric distances including posterior end to kinetoplast (PK), posterior end to midnucleus (PN), total length including flagellum (TL) and free flagellum (F) were measured using micrometry. Hematological parameters including differential and total W.B.C.counts and total R.B.C. counts were determined using autoanalyzer. Estimation of proteins was done using Lawry's method.

RESULT:

Prevalence of trypanosomal infection was observed in 70% of the sampled *Channa marulius*. Infected *Channa marulius* showed sluggishness in body movements, changes in body colour with presence of red or white patches, while blood samples of infected fish shows dark brown colour when stored at 4° C. Nonetheless, there was no significant difference in the length weight relationship of the infected and uninfected fish (Figure 1). Comparison of RBC count, blood proteins, blood urea and body proteins is as given in Figure 2. There was a decrease in the RBC count of infected fish as compared to the non-infected fish but this decrease in the RBC count was not significant. However, a significant decrease in the total proteins of blood ($t = 3.44$, $p < 0.05$) was observed. There was a decrease in the total muscle protein.

There was no significant difference in large lymphocytes count of infected and non-infected fish ($t = 0.343$, $P > 0.05$) while there were significantly more small lymphocyte count in infected fish than non-infected fish ($t = 1.32$, $P < 0.05$). We also found significantly less Neutrophils in infected than non-infected fish ($t = 4.38$, $P < 0.001$).

Eosinophils and monocyte count did not show significant difference between infected and non-infected fish. Estimations were carried out on the large and small lymphocyte counts in infections containing small, large and mixed(various) polymorphic forms of trypanosomes (Figure 3). It was observe that presence of large sized polymophic stages there was increase in small lymphocytes count while with increase in small sized polymorophic stages, there was increase in large lymphocytes count. Whereas in presence of various sized infected stages there was increase in large lymphocytes count.

DISCUSSION:

Percent prevalence of trypanosomes, morphology and new species from various other fishes were recorded by Wahul (1986). Pathogenesis of disease caused by trypanosomes is characterized by loss of weight and variety of anemia (Silva et. al., 1995). Present work shows decrease in weight of fish due to infection of trypanosomes. Also adhesion of trypanosomes to erythrocytes and erythophagocytosis was shown by Mario Luiz de la Rue, et.al, (1998). Present investigation also shows similar results.

Mario Luiz de la Rue. et.al (1998) showed hemolytic anemia with decrease in total R.B.C's count. In present study total R.B.C.'s count is very low in infected fishes as compared to non-infected fishes. But statistically it does not show considerable variation. Infection results, increase in lymphocyte count and decrease in neutrophils count in infected fishes than in non-infected fishes. This condition may occur as adaptation of parasite in body of host. Kidchakan Suparmattaya et.al (1999) recorded the hematological changes in *Channa marulius* showed that there is significant decrease in total

R.B.C's count and total W.B.C's .Thus hematological study shows changes in total R.B.C's, total W.B.C's, differential W.B.C's count and Hb count caused due to trypanosomiasis in *Channa marulius*. The neutrophils are remarkably decreased in infected fishes. It may be associated with the total W.B.C's count as other cell type of W.B.C's are not showing marked variation. Present investigation,(table-1) clearly shows that trypanosome infection to the economically important fish *Channa marulius* can detoriate the quality of fish and in turn an economical loss.

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Fig. 1: Images of infected fish *Channa marulius*.

Table 1.Parameters of infected and non-infected blood.

Parameter of Blood	Infected (Mean + 95%CI)	Non-Infected (Mean +95%CI)	t	p
RBC Count	3.8333(±0.6097)	4.3833(±0.5321)	-1.324	0.1923
Total Protein	0.0243(±0.0029)	0.0167(±0.0007)	-3.343	0.0096
Creatinine	0.0032(±0.0010)	0.0021(±0.0008)	1.6311	0.0687
Albumin	0.0055(±0.0020)	0.0047(±0.0019)	0.2975	0.2696
Urea	0.0076(±0.0191)	0.0492(±0.0182)	1.7599	0.0609
Body Muscle Proteins	0.6132(±0.0854)	0.6845(±0.5261)	0.8797	0.2381

Figure. 2. Comparision of different parameters in infected and non-infected *Channa marulius*. **All parameters except RBC count are standardized for unit body weight. Error bars are 95 % confidence intervals of the meansintervals of the means.**

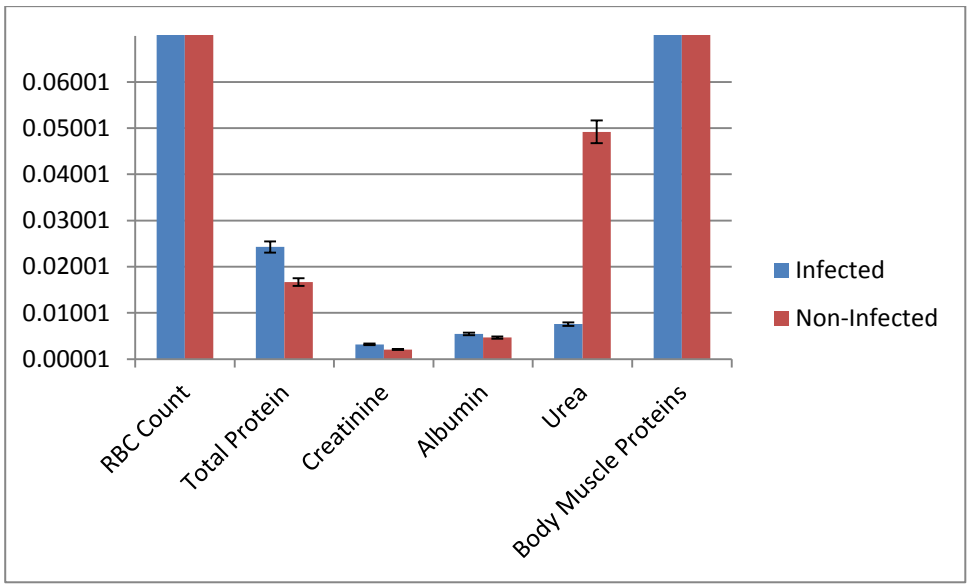

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Figure. 3. White blood cell counts in infected and non-infected *Channa marulius*. **Error bars are 95 % confidence intervals of the means.**